

**REMARKS**

Reconsideration is requested.

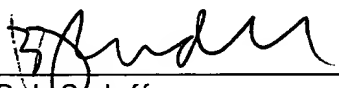
A copy of the Notification and Error Report dated July 17, 2002 is attached.

The attached paper and computer readable copies of the Sequence Listing are the same. No new matter has been added. A separate Statement to this effect is attached.

The present Amendment is believed to be completely responsive to the Notification however the Office is requested to advise the undersigned if anything further is required and allow additional time to respond.

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Respectfully submitted,

By   
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MARKED UP VERSION ATTACHED TO AMENDMENT IN  
SERIAL NO. 09/763,824

Marked up version of the paragraph on page 16, lines 10-11, is below:

Figure [5] 8 shows oligonucleotides (SEQ ID NOS 1-10, 11/36 and 12-33) used in the preparation of mutant enzymes of the invention.

Marked up version of the paragraph on page 17, lines 4-8, is below:

+Primer sequences:

W56:

5' - AAACAGGGACCCATATGGAAGACGC - 3' (SEQ ID NO: 34)

W57:

5' - AATTAAC TCGAGGAATTCGTCATCGCTGAATACAG - 3' (SEQ ID NO: 35)

Marked up version of the paragraph on page 19, lines 1-11, is below:

Example 3

Preparation of further triple mutant enzyme

The following primers were used to create the triple mutant T214A/I232A/E354K using a standard PCR reaction and with the pET23 plasmid with the T214A mutation as template:

CTGATTACACCCAAGGGGGATG (SEQ ID NO: 26) E354K-sense

CATCCCCCTTGGGTGTAATCAG (SEQ ID NO: 27) E354K-antisense

GCAATCAAATCGCTCCGGATACTGC (SEQ ID NO: 30) I232A-sense

GCAGTATCCGGAGCGATTTGATTGC (SEQ ID NO: 31) I232A-antisense.

Marked up version of the paragraph on page 19, lines 13-31, is below:

Example 4

Identification of thermostable 295 mutant

The F295 mutant was created using the error-prone PCR method described by Fromant et al., Analytical Biochemistry, vol 224, 347-353 (1995). The PCR conditions used were as follows:

0.5 µl (50 ng) plasmid pET23  
5.0 µl 10x KCI reaction buffer  
1 µl primer 1 - 60 pmoles of each primer  
1 µl primer 2  
1 µl Biotaq™ polymerase (5U)  
2 µl dNTPs, in mixture 35 mM dTTP, 12.5 mM dGTP, 22.5 mM dCTP, 14 mM dATP  
1.76 µl MgCl<sub>2</sub> (50 mM stock)  
1 µl MnCl<sub>2</sub> (25 mM stock) [final concentration in reaction = 3.26 mM]  
36.7 µl dH<sub>2</sub>O  
Primer 1 = 5' - AAACAGGGACCCATATGGAAGACGC - 3' (SEQ ID NO: 34)  
Primer 2 = 5' - AATTAACTCGAGGAATTCGTCATCGCTGAATACAG -3' (SEQ ID NO: 35)

Marked up version of the paragraph on page 21, lines 1-10, is below:

Example 5

Other mutants of the invention were produced by PCR using appropriate combinations of the oligonucleotides listed above as well as the following:

GAAAGGCCCGGCACCAGCCTATCCTCTAGAGG (SEQ ID NO: 5) F14A-sense  
CCTCTAGCGGATAGGCTGGTGCCGGGCCTTTC (SEQ ID NO: 6) F14A-antisense  
  
GAGATACGCCGCGGTTCTCTGG (SEQ ID NO: 9) L35A-sense  
CCAGGAACCGCGGCTATCTC (SEQ ID NO: 10) L35A-antisense